(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/042029 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US2003/035146

(22) International Filing Date: 4 November 2003 (04.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/423,760 5 November 2002 (05.11.2002) US

(71) Applicant *(for all designated States except US)*: **ISIS PHARMACEUTICALS, INC.** [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BAKER, Brenda, F.** [US/US]; 2147 Avenida Toronja, Carlsbad, CA 92009 (US). **ELDRUP, Anne, B.** [DK/US]; 1190 Encinitas Boulevard, Encinitas, CA 92024 (US). **MANOHARAN, Muthiah** [US/US]; 25 Circle Drive, Weston, MA 02493 (US). **BHAT, Balkrishen** [IN/US]; 911 Rosemary Avenue, Carlsbad, CA 92009 (US). **GRIFFEY, Richard** [US/US]; 360 Barsby Street, Vista, CA 92084 (US). **SWAYZE, Eric, E.** [US/US]; 7789 Palenque Street, Carlsbad, CA 92009 (US). **CROOKE, Stanley, T.** [US/US]; 3211 Piragua Street, Carlsbad, CA 92009 (US). **RAJEEV, Kallanthottathil, G.** [IN/US]; 1510 South Melrose Drive, Apt. #198, Vista, CA 92083 (US).

(74) Agents: **CALDWELL, John, W.** et al.; Woodcock Washburn LLP., One Liberty Place - 46th Floor, Philadelphia, PA 19103 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/042029 A2

(54) Title: OLIGOMERIC COMPOUNDS HAVING MODIFIED BASES FOR BINDING TO CYTOSINE AND URACIL OR THYMINE AND THEIR USE IN GENE MODULATION

(57) **Abstract:** Oligomer compositions comprising first and second oligomers are provided wherein at least a portion Of the second oligomer, at least a portion of the first oligomer is complementary to and capble of hybridizing to a selected target nucleic acid, and at least one of the first or second oligomers has a modified base for binding to a cytosine, uracil, or thymine base in the opposite strand. Oligonucleotide compositions are also provided comprising an oligomer complementartary To and capable of hybridizing to a selected target nucleic acid and at least one protein comprising at least a portion of an RNA-induced silencing complex (RISC), wherein the oligomer has a modified base for binding to a cytosine, uracil, or thymine base in the opposite strand.

OLIGOMERIC COMPOUNDS HAVING MODIFIED BASES FOR BINDING TO CYTOSINE AND URACIL OR THYMINE AND THEIR USE IN GENE MODULATION

Cross Reference to Related Applications

[0001] This application is a continuation in part of U.S. Provisional Patent Application Serial Number 60/423,760, filed November 5, 2002, and U.S. Patent Application Serial Number 10/078,949, filed February 20, 2002, which is a continuation of U.S. Patent Application Serial Number 09/479,783, filed January 7, 2000, which is a divisional of U.S. Patent Application Serial Number 08/870,608, filed June 6, 1997, now U.S. Patent Number 6,107,094, which is a continuation in part of U.S. Patent Application Serial Number 08/659,440, filed June 6, 1996, now U.S. Patent Number 5,898,031, each of which is incorporated herein by reference in its entirety.

Field of the Invention

[0002] The present invention provides modified oligomers that modulate gene expression via a RNA interference pathway. The oligomers of the invention include one or more modifications thereon resulting in differences in various physical properties and attributes compared to wild type nucleic acids. The modified oligomers are used alone or in compositions to modulate the targeted nucleic acids. In preferred embodiments of the invention, the modified oligomers contain at least one cytosine (C) and uracil (U) or thymine (T) modified binding base.

Background of the Invention

[0003] In many species, introduction of double-stranded RNA (dsRNA) induces potent and specific gene silencing. This phenomenon occurs in both plants and animals and has roles in viral defense and transposon silencing mechanisms. This phenomenon was originally described

more than a decade ago by researchers working with the petunia flower. While trying to deepen the purple color of these flowers, Jorgensen et al. introduced a pigment-producing gene under the control of a powerful promoter. Instead of the expected deep purple color, many of the flowers appeared variegated or even white. Jorgensen named the observed phenomenon "cosuppression", since the expression of both the introduced gene and the homologous endogenous gene was suppressed (Napoli et al., *Plant Cell,* **1990,** 2, 279-289; Jorgensen et al., *Plant Mol. Biol.*, **1996,** 31, 957-973).

[0004] Cosuppression has since been found to occur in many species of plants, fungi, and has been particularly well characterized in Neurospora crassa, where it is known as "quelling" (Cogoni and Macino, *Genes Dev.* **2000,** 10, 638-643; Guru, *Nature,* **2000,** 404, 804-808).

[0005] The first evidence that dsRNA could lead to gene silencing in animals came from work in the nematode, *Caenorhabditis elegans*. In 1995, researchers Guo and Kemphues were attempting to use antisense RNA to shut down expression of the par-1 gene in order to assess its function. As expected, injection of the antisense RNA disrupted expression of par-1, but quizzically, injection of the sense-strand control also disrupted expression (Guo and Kempheus, *Cell,* **1995,** 81, 611-620). This result was a puzzle until Fire et al. injected dsRNA (a mixture of both sense and antisense strands) into *C. elegans*. This injection resulted in much more efficient silencing than injection of either the sense or the antisense strands alone. Injection of just a few molecules of dsRNA per cell was sufficient to completely silence the homologous gene's expression. Furthermore, injection of dsRNA into the gut of the worm caused gene silencing not only throughout the worm, but also in first generation offspring (Fire et al., *Nature,* **1998,** 391, 806-811).

[0006] The potency of this phenomenon led Timmons and Fire to explore the limits of the dsRNA effects by feeding nematodes bacteria that had been engineered to express dsRNA homologous to the C. elegans unc-22 gene. Surprisingly, these worms developed an unc-22 null-like phenotype (Timmons and Fire, *Nature* **1998,** 395, 854; Timmons et al., *Gene,* **2001,** 263, 103-112). Further work showed that soaking worms in dsRNA was also able to induce silencing (Tabara et al., *Science,* **1998,** 282, 430-431). PCT publication WO 01/48183 discloses methods of inhibiting expression of a target gene in a nematode worm involving feeding to the worm a food organism which is capable of producing a double-stranded RNA structure having a nucleotide sequence substantially identical to a portion of the target gene following ingestion of

the food organism by the nematode, or by introducing a DNA capable of producing the double-stranded RNA structure (Bogaert et al., 2001).

[0007] The posttranscriptional gene silencing defined in *Caenorhabditis elegans* resulting from exposure to double-stranded RNA (dsRNA) has since been designated as RNA interference (RNAi). This term has come to generalize all forms of gene silencing involving dsRNA leading to the sequence-specific reduction of endogenous targeted mRNA levels; unlike co-suppression, in which transgenic DNA leads to silencing of both the transgene and the endogenous gene.

[0008] Introduction of exogenous double-stranded RNA (dsRNA) into *Caenorhabditis elegans* has been shown to specifically and potently disrupt the activity of genes containing homologous sequences. Montgomery et al. suggests that the primary interference affects of dsRNA are post-transcriptional. This conclusion being derived from examination of the primary DNA sequence after dsRNA-mediated interference and a finding of no evidence of alterations, followed by studies involving alteration of an upstream operon having no effect on the activity of its downstream gene. These results argue against an effect on initiation or elongation of transcription. Finally using in situ hybridization they observed that dsRNA-mediated interference produced a substantial, although not complete, reduction in accumulation of nascent transcripts in the nucleus, while cytoplasmic accumulation of transcripts was virtually eliminated. These results indicate that the endogenous mRNA is the primary target for interference and suggest a mechanism that degrades the targeted mRNA before translation can occur. It was also found that this mechanism is not dependent on the SMG system, an mRNA surveillance system in *C. elegans* responsible for targeting and destroying aberrant messages. The authors further suggest a model of how dsRNA might function as a catalytic mechanism to target homologous mRNAs for degradation. (Montgomery et al., *Proc. Natl. Acad. Sci. U S A,* **1998**, *95*, 15502-15507).

[0009] Recently, the development of a cell-free system from syncytial blastoderm Drosophila embryos, which recapitulates many of the features of RNAi, has been reported. The interference observed in this reaction is sequence specific, is promoted by dsRNA but not single-stranded RNA, functions by specific mRNA degradation, and requires a minimum length of dsRNA. Furthermore, preincubation of dsRNA potentiates its activity demonstrating that RNAi can be mediated by sequence-specific processes in soluble reactions (Tuschl et al., *Genes Dev.*, **1999**, *13*, 3191-3197).

[0010] In subsequent experiments, Tuschl et al, using the Drosophila in vitro system, demonstrated that 21- and 22-nt RNA fragments are the sequence-specific mediators of RNAi. These fragments, which they termed short interfering RNAs (siRNAs), were shown to be generated by an RNase III-like processing reaction from long dsRNA. They also showed that chemically synthesized siRNA duplexes with overhanging 3' ends mediate efficient target RNA cleavage in the Drosophila lysate, and that the cleavage site is located near the center of the region spanned by the guiding siRNA. In addition, they suggest that the direction of dsRNA processing determines whether sense or antisense target RNA can be cleaved by the siRNA-protein complex (Elbashir et al., *Genes Dev.*, **2001**, *15*, 188-200). Further characterization of the suppression of expression of endogenous and heterologous genes caused by the 21-23 nucleotide siRNAs have been investigated in several mammalian cell lines, including human embryonic kidney (293) and HeLa cells (Elbashir et al., *Nature*, **2001**, *411*, 494-498).

[0011] The Drosophila embryo extract system has been exploited, using green fluorescent protein and luciferase tagged siRNAs, to demonstrate that siRNAs can serve as primers to transform the target mRNA into dsRNA. The nascent dsRNA is degraded to eliminate the incorporated target mRNA while generating new siRNAs in a cycle of dsRNA synthesis and degradation. Evidence is also presented that mRNA-dependent siRNA incorporation to form dsRNA is carried out by an RNA-dependent RNA polymerase activity (RdRP) (Lipardi et al., *Cell*, **2001**, *107*, 297-307).

[0012] The involvement of an RNA-directed RNA polymerase and siRNA primers as reported by Lipardi et al. (Lipardi et al., *Cell*, **2001**, *107*, 297-307) is one of the many intriguing features of gene silencing by RNA interference. This suggests an apparent catalytic nature to the phenomenon. New biochemical and genetic evidence reported by Nishikura et al. also shows that an RNA-directed RNA polymerase chain reaction, primed by siRNA, amplifies the interference caused by a small amount of "trigger" dsRNA (Nishikura, *Cell*, **2001**, *107*, 415-418).

[0013] Investigating the role of "trigger" RNA amplification during RNA interference (RNAi) in *Caenorhabditis elegans*, Sijen et al revealed a substantial fraction of siRNAs that cannot derive directly from input dsRNA. Instead, a population of siRNAs (termed secondary siRNAs) appeared to derive from the action of the previously reported cellular RNA-directed RNA polymerase (RdRP) on mRNAs that are being targeted by the RNAi mechanism. The distribution of secondary siRNAs exhibited a distinct polarity (5'-3'; on the antisense strand), suggesting a cyclic amplification process in which RdRP is primed by existing siRNAs. This

amplification mechanism substantially augmented the potency of RNAi-based surveillance, while ensuring that the RNAi machinery will focus on expressed mRNAs (Sijen et al., *Cell,* **2001**, *107*, 465-476).

[0014] Most recently, Tijsterman et al. have shown that, in fact, single-stranded RNA oligomers of antisense polarity can be potent inducers of gene silencing. As is the case for co-suppression, they showed that antisense RNAs act independently of the RNAi genes rde-1 and rde-4 but require the mutator/RNAi gene mut-7 and a putative DEAD box RNA helicase, mut-14. According to the authors, their data favor the hypothesis that gene silencing is accomplished by RNA primer extension using the mRNA as template, leading to dsRNA that is subsequently degraded suggesting that single-stranded RNA oligomers are ultimately responsible for the RNAi phenomenon (Tijsterman et al., *Science,* **2002**, *295*, 694-697).

[0015] Several recent publications have described the structural requirements for the dsRNA trigger required for RNAi activity. Recent reports have indicated that ideal dsRNA sequences are 21nt in length containing 2 nt 3'-end overhangs (Elbashir et al, EMBO (2001), 20, 6877-6887, Sabine Brantl, *Biochimica et Biophysica Acta*, **2002**, *1575*, 15-25.) In this system, substitution of the 4 nucleosides from the 3'-end with 2'-deoxynucleosides has been demonstrated to not affect activity. On the other hand, substitution with 2'-deoxynucleosides or 2'-OMe-nucleosides throughout the sequence (sense or antisense) was shown to be deleterious to RNAi activity.

[0016] Investigation of the structural requirements for RNA silencing in *C. elegans* has demonstrated modification of the internucleotide linkage (phosphorothioate) to not interfere with activity (Parrish *et al., Molecular Cell*, **2000**, *6*, 1077-1087.) It was also shown by Parrish *et al.*, that chemical modification like 2'-amino or 5-iodouridine are well tolerated in the sense strand but not the antisense strand of the dsRNA suggesting differing roles for the 2 strands in RNAi. Base modification such as guanine to inosine (where one hydrogen bond is lost) has been demonstrated to decrease RNAi activity independently of the position of the modification (sense or antisense). Some "position independent" loss of activity has been observed following the introduction of mismatches in the dsRNA trigger. Some types of modifications, for example introduction of sterically demanding bases such as 5-iodoU, have been shown to be deleterious to RNAi activity when positioned in the antisense strand, whereas modifications positioned in the sense strand were shown to be less detrimental to RNAi activity. As was the case for the 21 nt dsRNA sequences, RNA-DNA heteroduplexes did not serve as triggers for RNAi. However,

dsRNA containing 2'-F-2'-deoxynucleosides appeared to be efficient in triggering RNAi response independent of the position (sense or antisense) of the 2'-F-2'-deoxynucleosides.

[0017] In one study the reduction of gene expression was studied using electroporated dsRNA and a 25mer morpholino oligomer in post implantation mouse embryos (Mellitzer *et al., Mehanisms of Development*, **2002**, *118*, 57-63). The morpholino oligomer did show activity but was not as effective as the dsRNA.

[0018] A number of PCT applications have recently been published that relate to the RNAi phenomenon. These include: PCT publication WO 00/44895; PCT publication WO 00/49035; PCT publication WO 00/63364; PCT publication WO 01/36641; PCT publication WO 01/36646; PCT publication WO 99/32619; PCT publication WO 00/44914; PCT publication WO 01/29058; and PCT publication WO 01/75164.

[0019] U.S. patents 5,898,031 and 6,107,094, each of which is commonly owned with this application and each of which is herein incorporated by reference, describe certain oligonucleotide having RNA like properties. When hybridized with RNA, these oligonucleotides serve as substrates for a dsRNase enzyme with resultant cleavage of the RNA by the enzyme.

[0020] In another recently published paper (Martinez *et al., Cell*, **2002**, *110*, 563-574) it was shown that single stranded as well as double stranded siRNA resides in the RNA-induced silencing complex (RISC) together with elF2C1 and elf2C2 (human GERp950) Argonaute proteins. The activity of 5'-phosphorylated single stranded siRNA was comparable to the double stranded siRNA in the system studied. In a related study, the inclusion of a 5'-phosphate moiety was shown to enhance activity of siRNA's in vivo in Drosophilia embryos (Boutla, et al., Curr. Biol., 2001, 11, 1776-1780). In another study, it was reported that the 5'-phosphate was required for siRNA function in human HeLa cells (Schwarz *et al., Molecular Cell*, **2002**, *10*, 537-548).

[0021] In yet another recently published paper (Chiu *et al., Molecular Cell*, **2002**, *10*, 549-561) it was shown that the 5'-hydroxyl group of the siRNA is essential as it is phosphorylated for activity while the 3'-hydroxyl group is not essential and tolerates substitute groups such as biotin. It was further shown that bulge structures in one or both of the sense or antisense strands either abolished or severely lowered the activity relative to the unmodified siRNA duplex. Also shown was severe lowering of activity when psoralen was used to cross link an siRNA duplex.

[0022] Like the RNAse H pathway, the RNA interference pathway for modulation of gene expression is an effective means for modulating the levels of specific gene products and, thus, would be useful in a number of therapeutic, diagnostic, and research applications involving

gene silencing. The present invention therefore provides oligomeric compounds useful for modulating gene expression pathways, including those relying on mechanisms of action such as RNA interference and dsRNA enzymes, as well as antisense and non-antisense mechanisms. One having skill in the art, once armed with this disclosure will be able, without undue experimentation, to identify preferred oligonucleotide compounds for these uses.

Summary of the Invention

[0023] In certain aspects, the invention relates to oligomer compositions comprising a first oligomer and a second oligomer in which at least a portion of the first oligomer is capable of hybridizing with at least a portion of the second oligomer, and at least a portion of the first oligomer is complementary to and capable of hybridizing to a selected target nucleic acid. At least one of the first or second oligomers includes at least one C and U or T modified binding base.

[0024] In certain other embodiments, the invention is directed to oligonucleotide/protein compositions comprising an oligomer complementary to and capable of hybridizing to a selected target nucleic acid, and at least one protein comprising at least a portion of a RNA-induced silencing complex (RISC). The oligomer includes at least one C and U or T modified binding base.

[0025] In other aspects, the invention relates to oligomers having at least a first region and a second region where the first region of the oligomer is complementary to and is capable of hybridizing with the second region of the oligomer, and at least a portion of the oligomer is complementary to and is capable of hybridizing to a selected target nucleic acid. The oligomer further includes at least one C and U or T modified binding base.

[0026] Also provided by the present invention are pharmaceutical compositions comprising any of the above compositions or oligomeric compounds and a pharmaceutically acceptable carrier.

[0027] Methods for modulating the expression of a target nucleic acid in a cell are also provided, wherein the methods comprise contacting the cell with any of the above compositions or oligomeric compounds.

[0028] Methods of treating or preventing a disease or condition associated with a target nucleic acid are also provided, wherein the methods comprise administering to a patient having

or predisposed to the disease or condition a therapeutically effective amount of any of the above compositions or oligomeric compounds.

Detailed Description of the Invention

[0029] The present invention provides oligomeric compounds useful in the modulation of gene expression. Although not intending to be bound by theory, oligomeric compounds of the invention are believed to modulate gene expression by hybridizing to a nucleic acid target resulting in loss of normal function of the target nucleic acid. As used herein, the term "target nucleic acid" or "nucleic acid target" is used for convenience to encompass any nucleic acid capable of being targeted including without limitation DNA, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA. In a preferred embodiment of this invention modulation of gene expression is effected via modulation of a RNA associated with the particular gene RNA.

[0030] The invention provides for modulation of a target nucleic acid that is a messenger RNA. The messenger RNA is degraded by the RNA interference mechanism as well as other mechanisms in which double stranded RNA/RNA structures are recognized and degraded, cleaved or otherwise rendered inoperable.

[0031] The functions of RNA to be interfered with can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include functions such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA. In the context of the present invention, "modulation" and "modulation of expression" mean either an increase (stimulation) or a decrease (inhibition) in the amount or levels of a nucleic acid molecule encoding the gene, e.g., DNA or RNA. Inhibition is often the preferred form of modulation of expression and mRNA is often a preferred target nucleic acid.

Compounds of the Invention

[0032] In certain aspects, the invention relates to oligomeric compounds that comprise at least one nucleotide containing a modified base. These modified bases are bases that will bind or hybridize to either an "C" base, i.e., an cytosine base on an cytidine nucleoside, or a "U or T" base, i.e., a uracil or thymine base on a uracil or thymidine nucleoside. Since these modified bases will bind to either a C base or a U or T base, for the purposes of this specification and the claims attached hereto the modified bases of the invention are identified as "C and U or T modified binding bases." Binding is meant in a Watson/Crick, Hoogsteen or reversed Hoogsteen like sense wherein one or more hydrogen bonds are formed between two bases forming a pair of complementary bases.

[0033] Excluded from the definition of C and U or T modified binding bases are the two natural puring bases A (adenine) and G (guanine). While the A and G bases bind to the C and U or T bases via hydrogen bonds in Watson/Crick type binding, they are not modified but exist in their natural form. Thus they are not C and U or T modified binding bases.

[0034] For the purposes of this specification and the claims attached thereto, C and U or T modified binding base include synthetic or natural modified purine bases, extended purine bases, purine bases that are joined to sugar moieties in nucleotides via a carbon atom, i.e., C-purine base, and nine- membered heterocyclic rings having 3, 4, or 5 nitrogen atoms in the ring.

[0035] Modified purine bases include 1-deazapurines, 3-deazapurines, 7-deazapurines, 9-deazapurines, 2-azapurines, 4-azapurines, 5-azapurines, 6-azapurines, 8-azapurines, and combinations thereof. Modified purine bases further include 2-substituted-purines, 6-substituted-purines, 8-substituted-purines, 1-N-substituted-purines, 3-N-substituted-purines, 7-N-substituted purines, and combinations thereof. These and other modified pyrimidine bases have been described in the art and identified in greater detail below.

[0036] Extended purines include ring systems having three rings in the system that include a purine or a modified purine as one of the rings of the ring system. Extended purines also include multiple ring systems wherein a purine ring is covalently bonded to a further single ring or to multiple rings via a covalent bond between the purine ring and the other ring or multiple rings or via a linker extending from the purine ring to the other ring or multiple rings. These ring systems may also include one or more linear side groups that extend from the ring

system much like a tail. These and other extended purine bases have been described in the art and identified in greater detail below.

[0037] Purine bases that are joined to sugar via a carbon atom in the purine ring (as opposed to the N-9 nitrogen atom) are known in the art as C-purines. Various C-purine bases have been described in the art and are identified in greater detail below.

[0038] Nine-membered heterocyclic rings having 3, 4, or 5 nitrogen atoms in the ring include 5H-Pyrrolo[2,3-d]pyrimidine (7-deaza purine), 5H-Pyrrolo[3,2-d]pyrimidine (9-deaza purine), 3H-Pyrazolo[3,4-d]pyrimidine (7-deaza, 8-aza purine), 1H-Pyrazolo[4,3-d]pyrimidine (8-aza, 9-deaza purine), and 5H-Pyrrolo[3,4-d]pyrimidine (7,9-deaza, 8-aza purine). Various six-membered heterocyclic rings having 3, 4, or 5 nitrogen atoms have been described in the art and are identified in greater detail below.

[0039] Preferred compounds that comprise C and U or T modified binding bases include, but are not limited to, boronated purine bases; C-2 and C-6 modified purine bases; 3-deazapurines; 7-deazapurines; 7-deaza, 8-azapurines; 8-azapurines; C2 and C6 modified or C5/C6 bismodified purines wherein the modifications include halo, alkyl, amino, cationic moieties, detectable labels or other modifications; 7 and/or 8 modified purines. Further preferred compounds that comprise C and U or T modified binding bases include tricyclic modified purine bases and modified purines that include two six-membered rings.

Hybridization

[0040] In the context of this invention, "hybridization" means the pairing of complementary strands of oligomeric compounds. In the present invention, the preferred mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

[0041] An oligomeric compound of the invention is believed to specifically hybridize to the target nucleic acid and interfere with its normal function to cause a loss of activity. There is preferably a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target nucleic acid sequences under conditions in which specific

binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

[0042] In the context of the present invention the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which an oligomeric compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will vary with different circumstances and in the context of this invention; "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated.

[0043] "Complementary," as used herein, refers to the capacity for precise pairing of two nucleobases regardless of where the two are located. For example, if a nucleobase at a certain position of an oligomeric compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the target nucleic acid are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases that can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligonucleotide and a target nucleic acid.

[0044] It is understood in the art that the sequence of the oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligomeric compound may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). It is preferred that the oligomeric compounds of the present invention comprise at least 70% sequence complementarity to a target region within the target nucleic acid, more preferably that they comprise 90% sequence complementarity and even more preferably comprise 95% sequence complementarity to the target region within the target nucleic acid sequence to which they are targeted. For example, an oligomeric compound in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/042029 A3

(51) International Patent Classification[7]: C12Q 1/68, C12N 5/00, A01N 43/04, A61K 31/70, C07H 21/04

(21) International Application Number: PCT/US2003/035146

(22) International Filing Date: 4 November 2003 (04.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/423,760 5 November 2002 (05.11.2002) US

(71) Applicant *(for all designated States except US)*: **ISIS PHARMACEUTICALS, INC.** [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BAKER, Brenda, F.** [US/US]; 2147 Avenida Toronja, Carlsbad, CA 92009 (US). **ELDRUP, Anne, B.** [DK/US]; 1190 Encinitas Boulevard, Encinitas, CA 92024 (US). **MANOHARAN, Muthiah** [US/US]; 25 Circle Drive, Weston, MA 02493 (US). **BHAT, Balkrishen** [IN/US]; 911 Rosemary Avenue, Carlsbad, CA 92009 (US). **GRIFFEY, Richard** [US/US]; 360 Barsby Street, Vista, CA 92084 (US). **SWAYZE, Eric, E.** [US/US]; 7789 Palenque Street, Carlsbad, CA 92009 (US). **CROOKE, Stanley, T.** [US/US]; 3211 Piragua Street, Carlsbad, CA 92009 (US). **RAJEEV, Kallanthottathil, G.** [IN/US]; 1510 South Melrose Drive, Apt. #198, Vista, CA 92083 (US).

(74) Agents: **CALDWELL, John, W.** et al.; Woodcock Washburn LLP., One Liberty Place - 46th Floor, Philadelphia, PA 19103 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 24 February 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OLIGOMERS COMPRISING MODIFIED BASES FOR BINDING CYTOSINE AND URACIL OR THYMINE AND THEIR USE

(57) Abstract: Oligomer compositions comprising first and second oligomers are provided wherein at least a portion Of the second oligomer, at least a portion of the first oligomer is complementary to and capble of hybridizing to a selected target nucleic acid, and at least one of the first or second oligomers has a modified base for binding to a cytosine, uracil, or thymine base in the opposite strand. Oligonucleotide&protein compositions are also provided comprising an oligomer complementartary To and capable of hybridizing to a selected target nucleic acid and at least one protein comprising at least a portion of an RNA-induced silencing complex (RISC), wherein the oligomer has a modified base for binding to a cytosine, uracil, or thymine base in the opposite strand.

WO 2004/042029 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/35146

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; C12N 5/00; A01N 43/04; A61K 31/70; C07H 21/04
US CL : 435/6, 325, 375; 536/24.5; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 325, 375; 536/24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002/0160393 A1 (SYMONDS et al) 21 October 2002 (21.10.2002), see page 2.	1-64 / 65-66
Y	US 6,294,522 B1 (ZABLOCKI et al) 25 September 2001 (25.09.2001), column 3, lines 35-49.	1-64 / 65-66
Y	US 6,150,510 A (SEELA et al) 21 November 2000 (21.11.2000), see columns 23-25.	1-64 / 65-66

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
15 November 2004 (15.11.2004)

Date of mailing of the international search report
04 JAN 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Janet L. Epps-Ford, Ph.D.
Telephone No. 571-272-0547

INTERNATIONAL SEARCH REPORT

Continuation of B. FIELDS SEARCHED Item 3:
CAplus, Medline, Biosis, USPATfull, PCTfull, JPO, EPO, Derwent
search terms: 8-modified adenosine, modified adenosine or adenine or guanine or guanosine, rnai, dsRNA, siRNA